REMARKS

Reconsideration of this Application is respectfully requested.

The Examiner is requested to return an initialed PTO-1449 Form confirming that the Information Disclosure Statement filed on June 21, 2001 has been fully considered pursuant to MPEP § 609.

Upon entry of the present amendment, claims 9-20 will remain pending in the application. These amendments do not introduce new matter and their entry is respectfully requested.

In the Office Action of January 3, 2002, the Examiner set forth a number of grounds for rejection. These grounds are addressed individually and in detail below.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 5-8 stand rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth at pages 2-5 of the Office Action. The major issue that the Examiner raised is that the amount of direction or guidance was insufficient to allow those skilled in the art to practice the invention without undue experimentation.

The Examiner asserts that the instant claims are not enabled because the specification fails to show the experimental data regarding the expression of B7 gene in K562 cells before combining with lymphocytes. The Examiner further recites several review articles to argue that in the absence of working examples

from the specification and the prior art, one skilled in the art would resort to experimentation to navigate the obstacles to practice the invention, because the nature of the invention is unpredictable.

Applicants enclose the data to demonstrate the expression of B7-1 gene in K562 cells (See attached Figure). If the Examiner would like the data submitted in the form of a 37 C.F.R. § 1.132 Declaration, please contact the undersigned.

Briefly, K562 cells were transfected by electroporation with an expression vector encoding human B7-1CDNA according to the method described by Teshigawara et al. (Nucleic Acids, Res, 20:2607 (1962)). The transfectants were selected in the presence of neomyan and then cloned. Figure 1 shows the result of a FACS analysis of human B7-1 expressed in the cloned transfectants.

Furthermore, as admitted by the Examiner, the present specification discloses the procedure for separating lymphocyte subset-NK cells from the blood. The specification also discloses that the B7 gene, which is incorporated into an expression vector, is expressed by K562 class-I antigen deficient cancer cells derived from human chronic myelocytic leukemia. The specification further discloses that human lymphocytes and K562 cells expressing the B7 gene are combined and incubated in a medium containing IL-2, human serum, and mitomycin to stop the amplification of the K562 cells. It is submitted that the disclosure of the present specification, as confirmed by the above-noted data, would enable one skilled in the art to practice

the invention without undue experimentation.

According to In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988), there are many factors to be considered when determining whether the specification provides an enabling disclosure or whether any necessary experimentation is "undue". These factors include, but are not limited to:

(A) The breadth of the claims;

(B) The nature of the invention;

(C) The state of the prior art;

(D) The level of one of ordinary skill;

(E) The level of predictability in the art;

(F) The amount of direction provided by the inventor;

(G) The existence of working examples; and

(H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure.

In re Wands, 858 F.2d 731, 737, (Fed. Cir. 1988).

It is improper to conclude that a disclosure is not enabling based on an analysis of only one of the above factors while ignoring one or more of the others. The Examiner's analysis must consider all of the evidence related to each of these factors, and any conclusion of non-enablement must be based on the evidence as a whole. *Id* at 1404, 1407.

With regard to the above described factors (factors A-H), the instant invention claims a method for culturing anti-cancer lymphocytes *in vitro*, and a pharmaceutical composition containing such anti-cancer lymphocytes. Claims 5-14 are directed to a

method for producing such lymphocytes in vitro. Claims 1-21 are directly to a composition which contains anti-cancer lymphocytes obtained by incubating lymphocytes with cancer cells under conditions to amplify mainly NK cells, non-MHC-bound T cells, or MHC-bound killer T cells.

The nature of the invention relates to the use of molecular biology and biological techniques to overcome problems in the field of immunology.

In this field, a person of ordinary skill in the art typically has a Ph.D. or M.D., and some working experience in cell biology, protein chemistry, molecular biology and immunology.

As discussed above, the present specification clearly discloses a procedure for separating lymphocyte subset-NK cells from the blood. The specification also discloses that the B7 gene, which is incorporated into an expression vector, is expressed by K562 class I antigen deficient cancer cells derived from human chronic myelocytic leukemia. The specification further discloses that human lymphocytes and K562 cells expressing the B7 gene are combined and incubated in a medium containing IL-2, human serum, and mitomycin to stop the amplification of the K562 cells. Furthermore, Applicants have enclosed data to illustrate the expression of the B7-1 gene by K562 cells before combining with lymphocytes (see attached Figure).

As to the element of predictability, the Examiner takes a

position that the practitioner of the invention must be able to predict, a priori, to provide working examples specifically directed to using the claimed composition and method treating and preventing cancer. Applicants have obtained the following data in the clinic trial using the present invention:

Case 1: Male, 60 years old. Colon cancer, invasion to retropertonium. He received NK therapy of the present invention after surgery two years ago, Stage III). There is no relapse at present.

Case 2: Male, 63 years old. Malignant lymphoma. There has been no progression of the disease without chemotherapy for over two years.

Case 3: Female, 53 years old. Relapse of mammary cancer. Chemotherapy did not improve the disease. After chemotherapy, immunotherapy was done. Immunotherapy reduced tumor size, and there is no progression of the disease.

Case 4: Male, 78 years old. Hepatocellular carcinoma. Metastasis in lung. Immunotherapy reduced AFP to normal level, and metastasis in lung disappeared.

Case 5: Male, 55 years old. Rhabdomyosarcoma. Chemotherapy did not improve the disease. Immunotherapy reduced tumor size and there has been no progression for three years.

These preliminary data suggest that immunotherapy with large numbers of activated NK cells is very effective in treating cancer. If the Examiner would like the data submitted in the form of a 37 C.F.R. § 1.132 Declaration, please contact the

undersigned.

Based on the disclosure of the specification and based on the state of the art, one skilled in the art would know how to make anti-cancer lymphocytes. Furthermore, one skilled in the art should know how to practice the invention using standard immunotherapy technology.

Taken together, Applicants respectfully submit that based on the evidence regarding each of the above factors, the specification at the time the application was filed would have taught one skilled in the art how to make and/or use the full scope of the claimed invention without undue experimentation.

It is believed that the grounds for this rejection have been obviated, and therefore the rejection under 35 U.S.C. § 112, first paragraph, for alleged lack of enabling disclosure should be withdrawn.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 1-8 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for the reasons set forth on pages 5-6 of the outstanding Office Action.

Applicants have cancelled claims 1-8. Therefore, this rejection is now moot. Accordingly, it is believed that the grounds for this rejection have been obviated and may properly be withdrawn.

Rejections Under 35 U.S.C. § 103

Claims 1-4 stand rejected under 35 U.S.C. § 103 a as being unpatentable over EP 0690125 A2 Tadao and further in view of Martin-Fontecha et al. (1998), for the reasons set forth on pages 6-7 of the outstanding Office Action.

Applicants respectfully traverse the rejection. To establish a *prima facie* case of obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). "All words in a claim must be considered in judging the patentability of that claim against the prior art." *In re Wilson*, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970).

The present invention relates to a method for culturing lymphocytes *in vitro*, and the lymphocytes, which can multiply NK cells or non-MHC-bound or MHC-bound killer cells in combination with killer T cells specific to cancer antigen. The lymphocytes of the present invention can be obtained by culturing lymphocytes and a cell with the B7 gene expressed in a particular cancer cell, or with the B7 gene and a particular gene, such as a cancer-antigen gene, expressed therein or a cell with such a gene already expressed therein to multiply mainly NK cells or non-MHC-bound or MHC-bound killer T cells, and multiplying killer T cells specific to a cancer antigen together with the NK cells or the non-MCH-bound or MHC-bound killer T cells. The present invention further provides a method for culturing lymphocytes *in vitro*, and the lymphocytes which involve using the particular cancer cells,

which are deficient or low in the expression of a class I antigen.

In contrast, Tadao discloses inducing anti-tumor CTL activity by culturing lymphocytes with freshly isolated tumor tissue. Tadao does not disclose the use of tumor cell lines as a stimulator. In fact, Tadao emphasizes the use of lymphocytes and tumor tissue from the same subject (Tadao, claim 1). The objective of using lymphocytes and tumor tissue from the same subject is to obtain specific killing of the specific tumor cells in the subject. This actually teaches away from the concept of using a tumor cell line as a stimulator where the individual specificity is lost. Tadao does not disclose or suggest culturing NK cells.

Martin discloses that CD40 and B7-2 molecules can interact with receptors on NK cells other than CD40L, and CD28. Martin, however, does not disclose transfecting cancer cells that are deficient in expression of class-I antigen. Martin does not disclose or suggest culturing NK cells. Furthermore, Martin is silent about T cells. Applicants would like to call the Examiner's attention to the fact that NK cells reduce their cell damage-causing activities when they amplify. To avoid such reduction, it is necessary to remove T cells and other cells by purification of NK cells. This process can only be achieved by the present invention.

Neither Tadao nor Martin discloses or suggests the amplification of NK cells. One skilled in the art would not be

able to produce the present invention based on Tadao and Martin without undue experimentation. Thus, it is not obvious to one skilled in the art to derive the present invention from the prior art of record.

Therefore, the references of Tadao and Martin do not support a *prima facie* case of obviousness. The grounds for this rejection have been obviated and withdrawal of the 35 U.S.C. § 103 rejection is respectfully requested.

Accordingly, in view of the above amendments and remarks, reconsideration of the rejections and allowance of the pending claims of the present application is respectfully requested.

CONCLUSION

All of the stated grounds for rejection have been properly traversed, accommodated or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. It is believed that a full and complete response as been made to the outstanding Office Action and as such the present application is in condition for allowance.

Prompt and favorable consideration of this response is respectfully requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Ping Wang, M.D. (Reg. No. 48,328), at the telephone number of the undersigned, to conduct an interview in

an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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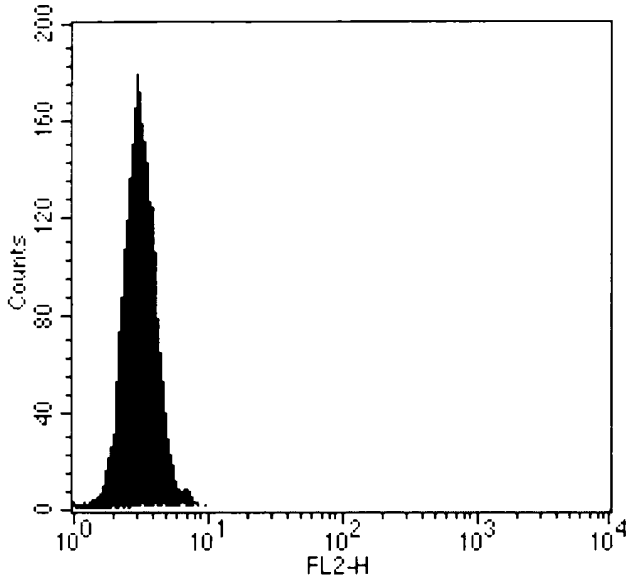
Attachment: Version With Markings Showing Changes Made
Figure

VERSION WITH MARKINGS SHOWING CHANGES MADE

IN THE CLAIMS:

Claims 1-8 have been cancelled.

Claims 9-20 have been added.



K562 cells were tranfected with the expression vector encoding human B7.1 cDNA.
These transfectants were selected in the presence of neomycin, and cloned.
The above figure shows the expression of human B7.1 antigen on the transfected K562 cells.
Left peak shows negative control (blue), and right peak(green) shows expression of human B7.1. These cells were analysed by FacsCan (Becton-Dickinson, Mountain View, California) after staining with phycaerythrin-conjugated anti-CD80 (B7.1) (Becton-Dickinson).